RESEARCH NOTE

SHELL MICROSTRUCTURAL RESPONSES OF *GEUKENSIA DEMISSA GRANOSISSIMA* (MOLLUSCA: BIVALVIA) TO CONTINUAL SUBMERGENCE

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In North America, the Atlantic ribbed mussel *Geukensia demissa* (Dillwyn, 1817) can be found intertidally in marshes from the Gulf of St. Lawrence to northeastern Florida (Abbott, 1974). There are two recognized subspecies of *G. demissa*, namely, *G. d. demissa* (Dillwyn, 1817) and *G. d. granosissima* (Sowerby, 1914) (Blackwell et *al.,* 1977). The latter is present along the Gulf Coast of Mississippi. Blackwell et *al.* (1977) suggested that the deposition of prisms found in the middle prismatic shell layer of the two subspecies was genetically regulated. Lutz and Rhoads (1978, 1980) and Lutz and Clark (1984) have shown seasonal and latitudinal variation in the inner shell layer of *G. demissa* inhabiting the Atlantic coast of North America. While juvenile *G. d. granosissima* are rarely found in subtidal habitats, adult ribbed mussels are never found subtidally (Heard, 1972). In this note, we report variation in growth of the internal shell nacre, induced by transplantation, of adult *G. d. granosissima* to a continuously submerged habitat in Ocean Springs, Mississippi.

Field experiments were carried out twice, a preliminary study in 1984 (3 March to 31 March) and a final study in 1985 (19 January to 23 February). Live mussels collected from emerged salt marsh (substratum normally exposed to air 50% of the time) fronting the Gulf Coast Research Laboratory, Ocean Springs, Mississippi, were divided into three groups of 20 mussels each. One group was shucked immediately and acted as a baseline for "normal" shell microstructure. Each of the other two groups was subdivided and placed into two separate wire mesh cages. One set (2 cages of 10 mussels each) was returned to the original site of collection [this habitat (emerged) was exposed to air during initial and final collection of mussels]. The other set was transplanted to a submerged area (substratum never exposed to air) less than 50 m seaward of the original collection site. Both sets of cages were set out within seven hours after initial collec-

tion. After about one month, shell microstructure of the caged mussels was examined by scanning electron microscopy and compared with baseline samples.

Adjusted 1985 tides for Biloxi Bay, Mississippi, indicated a tidal range from − 27 cm to + 58 cm. Predicted tides for 19 January 1985 were − 27 cm (0750 hr) and 58 cm (2108 hr). Predicted tides for 23 February 1985 were 30 cm (0119 hr), 9 cm (0807 hr), 27 cm (1300 hr) and 6 cm (2054 hr).

A warming trend in air and water (19.0-25.0ºC) occurred during the 1985 experiment (including freezing and temperatures from 20 to 23 January 1985). Salinity in Mississippi Sound varies from 0 to 16 ppt (Hackney and Cruz, 1982) and is usually low in winter and highest in March. Values we obtained correspond to reported values. Differences in the internal shell surface microstructures point to differences between regularly emerged and continually submerged habitats.

Areas of internal shell surface examined microstructurally are shown in plate 1. Based on 12 baseline mussels examined in January 1985, the internal shell surface of *Geukensia demissa granosissima* from Ocean Springs basically consists of the following shell microstructures: Starting from inside the pallial line, the "typical" nacre (Plate 1, Fig. A) composing the area towards the center of the shell (Plate 1, I_2) can be eroded to the extent that it appears homogeneous. This nacreous zone is adjacent to an area (Plate 1, I_2) composed of homogeneous [*sensu strictu (s.s.)*] microstructure whose granule sizes and shapes are less regular than those of the homogeneous (*s.s.*) microstructure in submerged mussels (Plate 1, Fig. B). A narrow transition zone leads to the pallial line composed of myostracum. This pallial myostracum (Plate 1, P) consists of short prisms (Plate 1, Fig. C) while the adductor scars (Plate 1, A) consist of tall prisms (Plate 1, Fig. D). Outside the pallial line, nacre (Plate 1,

Plate 1. Central line figure represents right valve of *Geukensia demissa granosissima* (internal shell surface with retractor scars omitted) surrounded by micrographs of corresponding shell microstructure (45° angle view of fractures with internal shell surfaces towards the top). Horizontal field width of micrographs = 16 μm. **A.** Nacre towards shell center (I_2). **B.** Homogeneous (*s.s.*) just inside pallial line (I_1). **C.** Short prisms composing pallial line (P). **D.** Tall prisms of adductor myostracum (A). **E.** Nacre between pallial line and outermost rim of shell (0).

Fig. E) again makes up the internal shell surface. The internal shell surface microstructure of the outermost rim (i.e. peripheral edge), however, can also be prismatic, blocky prismatic or homogeneous (*s.s.*). Variation of internal shell surface microstructure in the outermost rim can be a reflectoin of intermediate steps in the production of typically multiphasic outer shell layers.

We predicted that baseline and experimental emerged mussels would have similar internal shell microstructure unless the emerged mussels were "impinged" by the environment (over the one month duration of the experiment) or influenced by a cage effect. Indeed, these two groups were similar in internal shell structure with minor exceptions. Emerged mussels lacked the well formed nacre (mature tablets and growing nuclei) that were found in isolated pockets inside and outside the pallial line of baseline mussels.

For the comparative study of internal shell surface microstructures of emerged verus submerged mussels, only mussels of similar lengths (about 50 mm) were used. The main difference between emerged and submerged mussels in 1984 (limited sample) was in the posterior region of the shell outside the pallial line (Plate 2, Figs. A-B). Sizes and shapes of tablets in submerged mussels (Plate 2, Fig. A) were different from those of emerged mussels (Plate 2, Fig. B). Tablets of the former were elongated along one axis.

The 1985 transplantation experiment yielded greater internal shell surface microstructural differences between emerged and submerged mussels (Table 1). Relevant results presented in table 1 were based on examination of 10 valves of 10 individuals for each of the emerged and submerged

mussels.

Some emerged mussels had elevated borders of continuous ridges, beads (Plate 2, Fig. C) or granules that partially or completely surrounded one or more tablets along their 001 faces. These circumferential ridges resemble those structures attributed to shell formation and growth in *Pinctada martensii* (Dunker) (Wada, 1960, 1961), ring nacre of *Mytilimeria nuttalli* Conrad and *Lyonsia californica* Conrad (Prezant, 1981) and those attributed to shell dissolution in *Geukensia demissa* (Wilkes and Crenshaw, 1979; Rhoads and Lutz, 1980). Emerged mussels that exhibited these shell microstructures have fragmented and pitted tablets predominating in their internal shell surface (Table 1). The predominance of erosive remnants of nacre, both inside and outside the pallial line of emerged mussels (Table 1), indicates shell dissolution. Contrary to expectations, the warming trend in the weather inhibited shell formation in emerged mussels (absence of crystal nuclei, growing tablets and smooth surfaced tablets, etc.). Possibly a short cold spell following the day mussels were transplanted could have increased stress associated with the emerged habitat. One could speculate that the circumferential beads (Plate 2, Fig. C) are anlages to mature microstructures if one assumes that the emerged mussels were at a stage of recovery from shell dissolution (shell formation being initiated in response to changing

Table 1. Internal shell surface microstructures of *Geukensia demissa granosissima* after field experiment (1985) (– = absent, + to + + + + = degree of presence of microstructure in internal shell, where + = 1-25%, + + = 26-50%, + + + = 51-75%, + + + + = 76-100%).

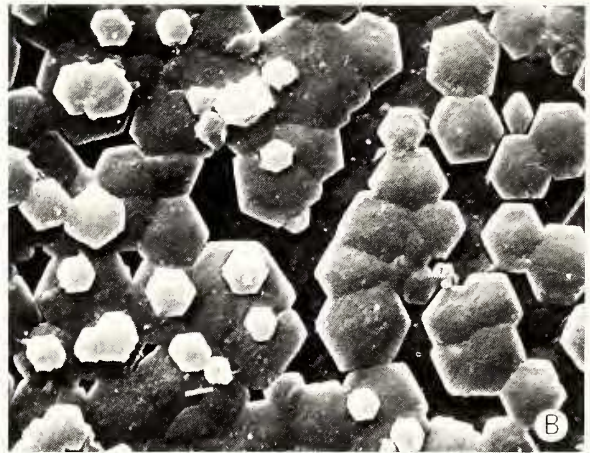|	Emerged Mussels	Submerged Mussels
A. OUTSIDE THE PALLIAL LINE		
1. anterior region		
—crystal nuclei and growing tablets	–	+
—smooth surface tablets	–	+
—pitted tablets	+ + + +	+
—ridged, beaded and granulated tablets	+	–
2. posterior region		
—crystal nuclei and growing tablets	–	+
—smooth surface tablets	–	+
—pitted tablets	+ + + +	+
—ridged, beaded and granulated tablets	–	–
B. INSIDE THE PALLIAL LINE		
1. anterior region		
—erosive remnants of nacre	+ + + +	+ +
—homogeneous (granules shape and size)	variable	uniform
2. posterior region		
—erosive remnants of nacre	+ + + +	+ +
—homogeneous (granules shape and size)	variable	uniform

Plate 2. A. Internal shell surface consists of elongated solitary and fusing polygonal tablets (Posterior region of submerged mussels, area O, March 1984). Horizontal field width = 22.8 μm. **B.** Internal shell surface consists of typical hexagonal tablet in various states of fusion (Posterior region of emerged mussels, area O, March 1984). Horizontal field width = 22.8 μm. **C.** Internal shell surface consists of peripherally beaded tablets (Anterior region of emerged mussel, area O, February 1985). Horizontal field width = 22.8 μm.

stressful to a more favorable condition). However, based on the overall picture and the presence of irregular pittings on the organic matrices where these structures were observed, we conclude that they are the result of incomplete dissolution.

Homogeneous (s.s.) internal shell surface microstructures in emerged mussels consisted of variably shaped granules, while those of submerged mussels consisted of uniformly shaped granules (Plate 1, Fig. B).

The uniformity of granule size and shape of homogeneous microstructure in the submerged mussels could be the result of well regulated formation. The assumption that shell formation is occurring in the submerged mussels is also supported by the presence of crystal nuclei and smooth surfaced tablets (Table 1) and apparent organic formations between and over tablets.

Mussels used in this experiment were taken from the same place at the same time. This assumes similarity of previous environmental influence at the start of the experiment. Furthermore, since the mussels utilized in this study were of similar size, variability due to age differences should be negligible. Growth rate of Geukensia demissa is higher along the marsh edge than in the higher marsh (Bertness and Grosholz, 1985). This, together with our observations, led us to hypothesize here that the submerged habitat is more stable, if not throughout the lifetime of the mussels, at least in this experiment. The surrounding water presumably acted as a buffer against severe weather variation. Continuous presence of water also insured access to food and nutrients and ready elimination of unwanted metabolic by-products. Normally, adult marsh mussels never occur subtidally; perhaps this is a reflection of blue crab or other predatory activities upon juvenile mussels (Bertness and Grosholz, 1985). In our experiments submerged mussels were protected from predators by cages. Continuous submergence of these protected mussels stimulated shell deposition and minimized shell dissolution.

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